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Art IV.—*Australian Ambrosia Fungi.*

(*Leptographium Lundbergii* Lagerberg et Melin, and *Endomycopsis* spp. Dekker.)

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(communicated by Dr. Ethel McLennan).

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Abstract.

1. The fungus *Leptographium Lundbergii* and two species of sporogenous yeasts belonging to the genus *Endomycopsis* were isolated constantly from the tunnels of the Australian ambrosia beetle, *Platypus subgranosus* in Myrtle beech, *Nothofagus Cunninghamii* and two other Australian timbers.

2. The characteristic features of *L. Lundbergii* are described in detail and compared with those of ambrosia fungi studied by other workers. The conclusion is reached that ambrosia fungi from different parts of the world belong to the same genus *Leptographium* and probably in many cases to the above-mentioned species. The first name associated with an ambrosia fungus was that of *Monilia candida* Hartig, but reasons are given why the fungus should not be placed in this genus.

3. The work of other authors connecting the conidial stage *Leptographium Lundbergii* with the ascigerous stage *Ceratostomella* is discussed and an affinity suggested between the Australian form and the species *Ceratostomella ips*.

4. The two species of *Endomycopsis* are described as Forms A and B. The frequent association of yeasts with wood-inhabiting beetles is mentioned and the question whether they serve the beetles directly as food or assist indirectly by stimulating the growth of the other fungus is discussed.

Contents.

Introduction.

It has been recognized for the past century that beetles belonging to the group of wood-boring insects known as Ambrosia beetles, cultivate in their galleries a fungous crop upon which they, and their larvae, subsequently feed. The beetles themselves have received considerable attention and have been described accurately, whereas comparatively little mycological work has been done on the associated fungi.

Specimens of the timber of myrtle beech, *Nothofagus Cunninghamii,* which had been attacked by the Ambrosia beetle *Platypus subgranosus,* were submitted to the Botany Department of the Melbourne University and many cultures were made from the wood immediately surrounding the galleries of the beetle, and resulted in the constant isolation of one fungus and two types of sporogenous yeasts. The fungus was identified as *Leptographium Lundbergii,* Lagerberg et Melin, and the yeasts as species of the genus *Endomycopsis* Dekker. Cultures from the beetles themselves and from larvae taken from the galleries also gave the same forms.

Isolations from the galleries of the beetle in two other Australian timbers, Mountain Grey Gum, *Eucalyptus goniocalyx* and Mountain Ash, *E. regnans,* helped to confirm the constant association of *Leptographium Lundbergii* and the *Endomycopsis* spp. with *Platypus subgranosus.*

Method of Isolation.

In making isolations from the beetle galleries, a block of infested timber, preferably with larvae or beetles still present, was taken and sterilized superficially with mercuric chloride solution. The block was then split with a sterilized tomahawk in order to expose portion of the gallery or tunnel. Small slivers of the timber at the blackened edge of the gallery were removed with sterile chisel forceps and transferred to malt agar plates. The plates were left in the laboratory at room temperature during the warmer months of the year, but during the winter were incubated at 25°C. Appreciable growth of the associated organisms took place within four or five days.

Account of Leptographium Lundbergii.

Nomenclature.

Leptographium Lundbergii is the type species of the genus *Leptographium* Lagerberg et Melin, created and described by Lagerberg, Lundberg and Melin (14) for a fungus which they isolated from a trunk of *Pinus sylvestris* showing intense blue-staining. This organism is now known to be a common cause of blue-stain in conifers and it has also been recorded by Verrall (28) as a lesser staining fungus of hardwoods in America.

Lagerberg and Melin emphasized when they created the genus, as did Grosmann subsequently, that *Leptographium* was in all probability identical with the genus *Scopularia* Preuss. *Scopularia venusta* was the name given by Preuss (18) to a blue-staining fungus which he found on decorticated pinewood in 1851. No spore measurements or details were given and the conidiophores were apparently falsely pictured. They were shown to be branched in a penicillate fashion and the individual branches to be septated. However, the curious manner in which the bases of the branches were extended across the main stalk of the conidiophore in a collar-like fashion looks very unreal. Saccardo doubted the fidelity of the reproduction, and as the fungus was not found again, it seems better to adhere to the later genus *Leptographium* rather than to the somewhat uncertain *Scopularia*.

Goidanich (7), a later worker, preferred the use of *Scopularia* to *Leptographium* and transferred *L. Lundbergii* to that genus.

Growth Characteristics.

On malt agar, the fungus is fast growing, covering a 9 cm. Petri dish in four to five days. At first, it is sparse and white with very long aerial hyphae. In an inverted petri dish culture, these aerial hyphae reach right down from the surface of the agar to the lid as fine strands. In the majority of cultures, the white mycelium soon acquires a rather powdery appearance due to the formation of abundant conidia.

As the culture ages, the mycelium gradually darkens, while the medium changes colour more rapidly and passes through various shades of brown, from Brussels Brown, Raw Umber, and Cinnamon Brown to Fuscous Black or Chaetura Black (Ridgeway Colour Standards and Colour Nomenclature). The aerial hyphae tend to flatten out very soon with the collection of drops of honey-coloured liquid and the surface of the culture appears moist or even sodden.

After a period of time, varying from about two weeks to two or more months, round yellowish-brown yeasty spots, up to 5 mm. in diameter, appear in many though not all of the cultures, and it is in these that the typical conidia and conidiophores of *Leptographium* are found clustered together.

After subculturing for some time, more aerial mycelium may be formed and the surface of the culture is then quite woolly, losing its yeasty or sodden appearance.

As the cultures commence to change in colour and become brown, microscopic examination shows the formation of yellow to dark-brown hyphae, twisted in loose strands, both in the aerial

and submerged mycelium. Very wide brown hyphae, 10μ or more in diameter, also make their appearance and are characteristic.

The conidia are hyaline and unicellular and are extremely variable in shape and size. Those formed in very young cultures are as a rule oval or nearly cylindrical and may be from about 2 to 16μ in length (fig. 1A). Those formed later on the mature

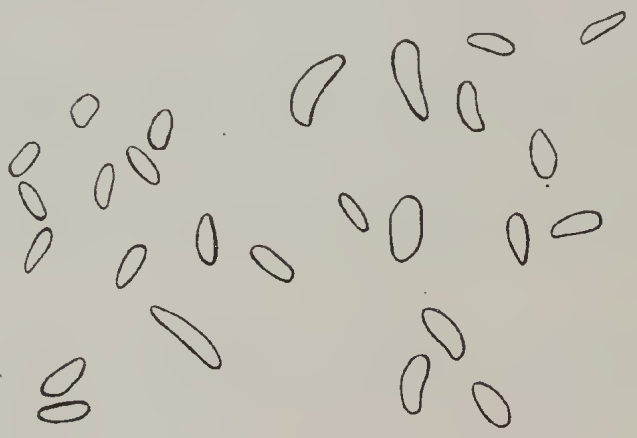

FIG. 1A.—Conidia from a young culture of *Leptographium Lundbergii*. × 960.

conidiophores are usually somewhat truncate and less variable in size, the average for 100 conidia being 8·9μ by 5·3μ with a range from 4 to 15μ by 3 to 7μ (fig. 1B).

FIG. 1B.—Conidia from a mature conidiophore in an old culture. × 960.

The mode of formation of the conidia varies considerably according to the age of the culture. At first, they are borne singly on the tips of the hyphae and on short branches along the

main hyphae (fig. 2). The conidia tend to adhere together in mucous and form small round heads. This can be readily observed if the fungus is grown on a clear medium, when the spore heads

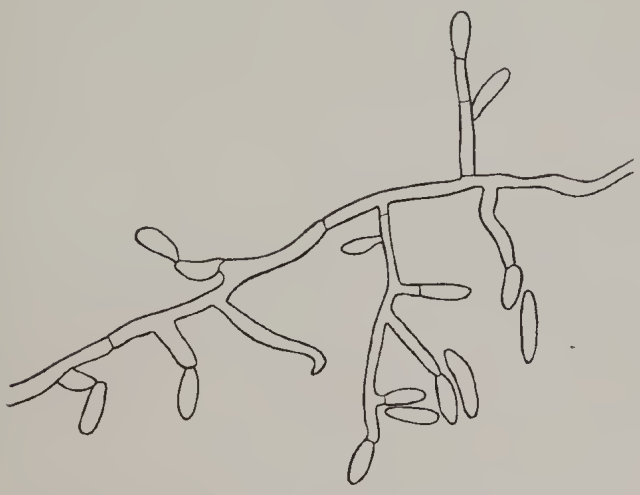

Fig. 2.—Early stage in conidial formation. × approx. 720.

can be seen in situ through the agar (fig. 3). In this stage, a strong resemblance to the genus *Cephalosporium* is shown, and if the later mode of conidial development were not observed, the

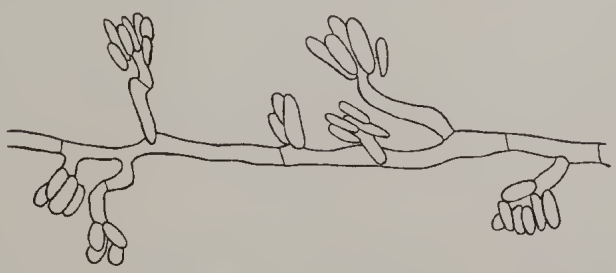

Fig. 3.—Cephalosporium stage of conidial formation. × approx. 720.

fungus could readily be placed in that genus. This stage will be referred to in future as the Cephalosporium stage. *Cephalosporium* Corda (Icon. Fung. III., II., 1839) is characterized by possessing unbranched conidiophores which arise as short lateral branches which are not swollen at the apex. The hyaline conidia arise singly at the tip of the conidiophore and are pushed to the side by the subsequent conidia without falling off, many holding together in a little mucous, forming a small spherical hyaline head.

As the culture ages, the Cephalosporium stage is passed, and conidia are constricted off from side branches which become more and more complexly branched, until eventually large brush-like heads are formed (figs. 4 and 5). The conidia are at first oval to somewhat rounded, but before being cut off from the

FIG. 4.—Simple branched conidiophores. × approx. 720.

conidiophore become more pear-shaped or truncate. The branches of the conidiophore are septated and at first hyaline, but on maturing become brown in colour towards the base. The septations of the mature conidiophore are close together and at times give a distinct monilioid appearance. The conidia normally remain hyaline, but very occasionally individual ones may become yellow or brown in colour.

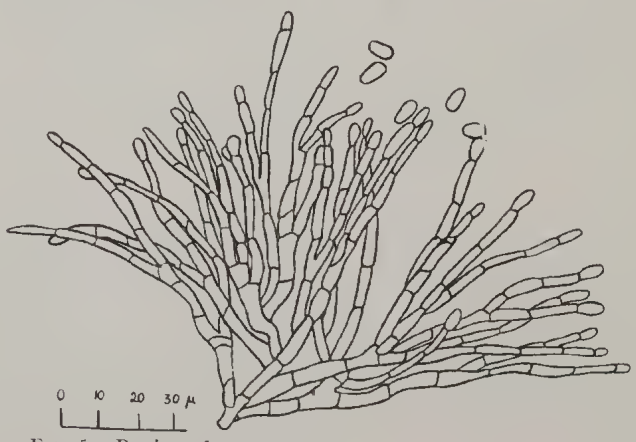

FIG. 5.—Portion of a mature conidiophore. × approx. 480.

The conidia frequently bud in a yeastlike fashion as soon as they are shed and produce a beaded or monilioid type of mycelium on the surface of the culture, giving rise to the yeasty or sodden appearance mentioned above. At times, this yeast-like budding is so profuse in a young culture, that the surface of the colony is flat and moist and faintly yellow or brown in colour and there is no formation of the long white aerial hyphae typical of most young cultures.

Isolations from the beetle galleries in Mountain Ash, *Eucalyptus regnans*, gave in addition to the typical fungus a much more slowly growing form of *L. Lundbergii*. This form was characterized by a complete lack of aerial mycelium and by the very early formation of typical *Leptographium* conidiophores in sulphur yellow mounds. The first formed conidia were more like the mature conidia in shape and size and not nearly as variable as those formed at first in cultures with more aerial mycelium.

Appearance in the Beetle Tunnels.

The appearance of the ambrosia fungi in the beetle tunnels in the wood has been described by most authors as a palisade of monilioid chains of cells showing glistening white when young but discolouring with age.

When sections of the tunnels of the Australian ambrosia beetle in myrtle beech are examined under the microscope, a similar condition is seen. If examined closely, however, it will be seen that the palisade consists of fairly wide septated hyphae, which are light-brown at the base and gradually pale off to the tip where a single hyaline, truncate conidium is borne. The septations give the appearance of moniliform chains, but actually only a single conidium is carried at the tip of each septated hypha. This condition is illustrated in Plate IV., figs. 1 and 2, and if the fungal layer is studied and compared with the illustration of Leach *et al* (16) of the ambrosia fungus in the tunnels of the beetle *Trypodendron retusum* in *Populus tremuloides*, the two will be seen to be strikingly similar. At this stage, the ambrosia fungus in the tunnels resembles very closely the apices of the ultimate branches of the mature conidiophores of *Leptographium Lundbergii*. The actual branching of the conidiophores is obscured because of the dense growth of the fungus around the edge of the tunnel.

Since the beetles and larvae are cropping continually at the fungal layer, this complete condition of the apices of the conidiophores with their terminal conidia forming a continuous palisade is not often to be found. More frequently, the short wide hyphae are cut off to about the level of the first septum and are seen with jagged ends while the conidia lie loosely about their bases. An

occasional long septated hypha complete with its terminal conidium projects forth into the cavity of the tunnel where it has been missed by the beetles. At other times less mature conidiophores are seen with narrower hyphae and more rounded conidia at the apex.

The wood adjacent to the tunnels of the beetles is distinctly blackened, giving the appearance that the tunnels have been made by plunging red-hot needles into the wood. The black stain does not spread more than about 1 mm. in a transverse direction or horizontal direction but may extend 10 mm. or more longitudinally from the edge of the tunnel. When sections of the blackened wood are examined, the wood vessels and fibres are seen to be densely packed with dark, much branched hyphae. The presence of these dark hyphae and the resultant staining of the timber around the tunnels lend support to the assumption that the ambrosia fungus is a wood-staining organism. Many yeast-like cells are also present lining the tunnels and at times it is difficult to distinguish between them and the immature conidia of *L. Lundbergii.*

The beetles and larvae keep the fungus closely cropped and the galleries remain clear in their presence. If, however, the timber is kept for a short time after the emergence of the insects, the conidia and yeast cells germinate and give rise to a headed monilioid type of mycelium. This stage is soon passed over and the tunnels rapidly become blocked with a tangle or plug of white mycelium.

Comparison with other Ambrosia Fungi.

Thomas Hartig (11) was the first to recognize the fungal nature of the ambrosia and in 1844 gave the name *Monilia candida* to the ambrosia fungus of the beetle *Xyleborus* (*Bostrychus*) *dispar.*

In 1897, Hubbard (13) discussed the ambrosia beetles of the United States and gave illustrations and descriptions of their respective fungi. Although he made no attempt to name or grow them in culture, he pointed out that they were specific and that only the most closely related species of beetle had the same food fungus. His illustrations are interesting and informative. The long septated conidiophores which he pictured for the ambrosia fungus of the beetle *Xyleborus pubescens* and that of *X. celsus* show a strong resemblance to those described above from the galleries of the Australian beetle, *Platypus subgranosus.* Hubbard observed that an umber brown discolouration tinged the base of the clustered "stems" of the ambrosia of *X. celsus,* but that their terminations were pellucid and filled with colourless protoplasmic granules. This point increases the resemblance to the terminal branches of the conidiophores of *Leptographium Lundbergii.*

He described the ambrosia of *Xyleborus xylographus* as consisting of short erect stems terminating in spherical conidia and pictured the so-called stems each with three or four septa. He stated that the freshly grown fungus is as colourless as crystal but that it is usually more or less stained greenish-yellow, sometimes resembling a coating of sublimed sulphur. Rumbold (19) in a paper on the association of blue-staining fungi with bark beetles in pines, mentioned Hubbard's work on ambrosia beetles and she too noticed the resemblance to *Leptographium Lundbergii,* pointing out that this description of Hubbard's " reminds one of the greenish-yellow clumps of conidia, which later are honey coloured, that characterize the test tube cultures of *Leptographium Lundbergii,* Lagerberg et Melin." The ambrosia fungus of *Xyleborus pubescens* as figured by Hubbard, reminded Rumbold of the conidiophores of either *Ceratostomella pini* or *C. ips,* although she stated that his illustration did not represent them exactly. This point is of interest in view of work to be mentioned later which connects species of *Ceratostomella* including *Ceratostomella penicillata* and *C. ips* with the imperfect stage of *Leptographium Lundbergii.*

Hubbard was of the opinion that the ambrosia fungi were specific and that only the most closely related species of beetle cultivated the same food fungus. However, it is possible that he observed the same species of fungus in different stages and did not recognize the relationship between them.

Schneider-Orelli (22) gave a more complete account of the ambrosia fungus of *Xyleborus dispar.* He stated that the walls of the beetle galleries were lined with hyaline, thin-walled, septated hyphae which swell out at the apex into a sphere; at later stages these spherical cells could be seen in long chains. He mentioned that in the galleries of another species, *Xyleborus saxeseni,* the cells remained single and were not seen in chains. According to Schneider-Orelli, the ambrosia fungus lost its monilial nature when grown in culture and became more truly mycelial. However, even the mycelial growth was characteristic, particularly with regard to the browning of the upper surface of the culture and the reddish-brown and ultimate black colouration of the medium. He did not observe a true spore stage in culture, and on this account did not give the fungus a definite name, not being completely satisfied with *Monilia candida* Hartig.

Three Californian Ambrosia beetles were described by Doane and Gilliland (5) in 1929 and brief references made to the ambrosia fungi associated with them. *Monarthrum scutellare* and *M. dctigerum* on oak were found to cultivate a fungus which they thought was probably a species of *Monilia.* They stated that in culture on alfalfa agar " the conidiophores grow from the prostrate mycelium and these bear branching hyphae which form

terminally continuous chains of conidia." As this is the extent of the description of the fungus in culture, it is difficult to compare it with forms examined by other workers.

Trotter (27) in 1934 examined the ambrosia fungus of a tropical *Xyleborus* species in the branches of *Brownea Grandiceps* from Ceylon. He observed a layer of short chains of sub-olivaceous, sterile, torulose, subglobose hyphae, 8-12μ in diameter, composed of two or more conidium-like segments of which the terminal one was the thickest. A second fungal layer above this consisted of a whitish mass of hyaline, variously shaped continuous conidia ranging from 8 to 4 by 35 to 7·5μ or even larger. When grown in culture, new conidia were produced of the type observed in nature, and, on the same mycelium, short branches with microconidia. Trotter considered the fungus to belong to an undescribed genus and named it *Ambrosiamyces zeylandicus* n. gen., n. sp. Leach was of the opinion that Trotter was dealing with a fungus completely unrelated to his or Hartig's ambrosia fungus. However, the chains of sub-olivaceous hyphae composed of two or more conidium-shaped segments, crowned by a layer of hyaline conidia are very suggestive of the picture presented by *Leptographium Lundbergii* in the tunnels of the Australian Ambrosia beetle, while the production of micro-conidia on short branches resembles the Cephalosporium stage of young cultures mentioned earlier, in which the first formed conidia are frequently very small and are seen in small heads. The possibility that Trotter was dealing with *L. Lundbergii* or a closely allied species does not therefore seem to be excluded.

More recent work on ambrosia fungus has been published by Leach, Hodson, Chilton, and Christensen (16). These authors gave a detailed description of the ambrosia fungus of two species of beetles, *Trypodendron betulae* on birch and *T. retusm* on aspen. These two beetles cultivate the same fungus which Leach and his co-workers considered to show enough resemblance to the ambrosia fungus of *Xyleborus dispar,* as described by Hartig and Schneider-Orelli to be placed in the same genus, though probably in a different species. On account of the confusion over the nomenclature of *Monilia candida,* they made no attempt to apply a new name, thinking that ambrosia fungi should be studied in more detail before their rightful place was decided. Pending further studies, they thought the fungi associated with the beetles *Trypodendron betulae* and *T. retusum* might be considered as strains of *Monilia candida* Hartig.

The ambrosia fungus of these *Trypodendron* species, when grown in culture, was at first hyaline but became brown with age and the medium was discoloured with a diffusible brown stain. At first sporulation was poor and only imperfect monilioid spores, that tended to remain attached and bud in situ, were formed. After repeated subculturing, variants that sporulated abundantly

and consistently were obtained. The spores were hyaline and averaged 11·38μ by 10·09μ in size, with a range of 6 to 17μ in length and 6 to 14μ in width. No reference was made to branched conidiophores, but the description of the fungus otherwise corresponds well with that of *Leptographium Lundbergii*. The authors did not observe the large yellow to brown yeasty patches formed by the typical *L. Lundbergii* conidiophores in culture, but as these are often produced only after a period of two months or more, and sometimes not at all, it is not surprising that their formation was overlooked. The spore measurements come within the range of *L. Lundbergii*. The appearance of the *Trypodendron* ambrosia fungus in the beetle galleries in aspen and that of the Australian beetle, *Platypus subgranosus* in myrtle beech can be seen to be identical, if a comparison is made of the figure in the paper by the above-mentioned workers and Plate IV., figs. 1 and 2 in the present paper.

Verrall (30) made a number of new species for the ambrosia fungi which he found in constant association with species of *Platypus, Pterocyclon,* and *Xyleborus*. *Cephalosporium pallidum* is the name which he gave to the ambrosia fungus of the beetle *Xyleborus affinis*. His description of the fungus is as follows:— "On malt agar, colonies are moderately slow growing, reaching 9 to 14 mm. in radius in six days at room temperature. The margins are usually appressed and hyaline while the rest of the colony is covered with a thin layer of hyaline, fluffy aerial mycelium which often becomes appressed with age except for isolated tufts. Aerial mycelium may be entirely lacking. Occasionally a slight brownish tinge develops in parts of old cultures. Yellowish yeasty mounds develop in ageing cultures. In the yeasty mounds, mycelium may be limited to pointed short celled hyphae projecting but shortly from the yeasty mass of conidia and monilioid cells. Compact helicoid hyphal formations were commonly observed in the filamentous mycelium.

Conidia germinate on malt agar by forming monilioid chains of cells which finally give rise to hyphae. Spore heads are formed relatively soon after germination. In culture, typical fruiting consists of cephalosporic heads of conidia protruding but slightly above the agar on erect or decumbent conidiophores. Conidiophores are generally unbranched and hyaline and terminate in one to ten or more hyaline unicellular conidia which are nearly spherical to slightly pear shaped, 7·6μ to 14·4μ long and 7·9μ to 14·0μ wide, averaging 10·8 by 10·4μ. When appreciable aerial mycelium occurs conidiophores elongate and branch. Sometimes the conidiophores are composed partly or totally of moniliform cells, particularly in the yeasty mounds. Occasionally buds were observed forming laterally on hyphae and monilioid chains of spores of irregular sizes and shapes were observed in the agar or protruding above it."

It has already been pointed out that cultures of *L. Lundbergii* pass through a Cephalosporium stage when young and that in this condition they might be mistaken for cultures of *Cephalosporium*. Moreover the mature septated conidiophores in the yeasty mounds have a distinctly monilioid appearance and are clustered so thickly together, that unless teased out and examined very closely, their complex branched nature is not readily observed. Altogether Verrall's description of *Cephalosporium pallidum* in culture shows such striking resemblances to *Leptographium Lundbergii*, that the justification for placing it in the genus *Cephalosporium* and his creation of a new species may be seriously questioned. However, his cultures are not available for comparison and it is therefore not possible to say that he was in reality dealing with *L. Lundbergii*. Verrall believed *Cephalosporium pallidum* to be related to *Monilia candida* Hartig. He pointed out, however that Schneider-Orelli, in describing Hartig's fungus, had made no mention of yellowish yeasty mounds or of cephalosporic heads.

Verrall created a second species of *Cephalosporium*, *C. luteum*, for the fungus which he found in association with the ambrosia beetle, *Xyleborus pecanis*. He did not observe yeasty mounds in cultures of his *C. luteum*, but he reports that the cultures were at first hyaline, though soon becoming sulphur yellow to light-brown, while the agar was stained a deep brown. Aerial mycelium was at first fluffy but became appressed with age. Spores were difficult to find, but when produced formed on simple or branched conidiophores, mostly singly, sometimes in heads of two or three spores. Once again, Verrall's fungus shows marked similarities to *L. Lundbergii*, and once more objection must be raised to his decision to place it in the genus *Cephalosporium*.

The ambrosia fungus associated with the beetles *Pterocyclon mali* and *P. fasciatum* was reported by Verrall to be *Monilia brunnea* n. sp. Cultures of this fungus were at first hyaline but became dark-brown with age and the original isolates were quite yeasty in appearance and consisted largely of monilioid chains of rounded cells that budded in situ. More mycelial growth developed with repeated culturing and small dark-brown mounds of monilial cells were seen in older cultures. These monilial cells were at times distinctly brown in old cultures and borne in simple or branched chains. Verrall expressed the opinion that his *Monilia brunnea* was similar to, but not the same fungus as that described by Leach *et al* (16), for the ambrosia of the beetles *Trypodendron betulae* and *T. retusae* already described above. In spite of the similarities he preferred to create a new species for it. It can be seen, however, that all his points fit into the picture of *Leptographium Lundbergii*, the fungus described in this paper as the ambrosia of *Platypus subgranosus*, and it seems probable that he was in reality dealing with the same fungus.

Connection with the Genus Monilia.

The striking similarities in the ambrosia fungi described by authors from different parts of the world and discussed above, seem to indicate that they are all closely related or that they actually belong to the same species. In each case, the resemblance to *Leptographium Lundbergii* has been pointed out and the inference to be drawn is that the ambrosia fungus of Hartig, and those of Schneider-Orelli, Leach *et al*, Trotter and Verrall can all be linked up together with the former genus if not all with the species *L. Lundbergii*.

Leptographium Lundbergii is an extremely variable fungus and presents very different appearances at the different stages of its growth. The young cultures in the Cephalosporium stage with their fluffy aerial mycelium might easily be considered to belong to a different genus from the older stages with their appressed light-brown coloured mycelium, darkened agar and typical *Leptographium* conidiophores in the yellow or brown yeasty mounds. Some isolates produce more aerial mycelium and sporulate less frequently than others. It is therefore quite probable that it should have been described under various names by different workers.

The first name given to an ambrosia fungus was that of *Monilia candida*, by Hartig in 1844. However, Schneider-Orelli pointed out that since the work of Hartig, the name *Monilia candida* had been used by Bonorden (3) for a different fungus, the yeast-like form now so well known in the literature of fermentation. Bonorden was apparently ignorant of Hartig's earlier use of the name for the ambrosia fungus. *Monilia candida* Hartig remained completely disregarded for a considerable time, so that any discussion of *Monilia candida* in mycological text books almost always refers to the Bonorden fungus. Although Hartig's fungus actually would have prior claim, the name *Monilia candida* is in such common use for Bonorden's fungus that much confusion would arise in any attempt to change it.

But in any case, the use of the name *Monilia candida* for the ambrosia fungus seems to be excluded. The genus *Monilia* Persoon is characterized by having conidiophores with dichotomous grape-like or irregular, sparing or frequent branching, bearing at the tips of the branches or on little blunt teeth near the tips the simple or branched chains of hyaline conidia. The conidia of the Australian ambrosia fungus are borne singly, not in chains and therefore it should not be placed in the genus *Monilia*. Provided the assumption is correct that other workers have mistaken the septated terminal branches of the conidiophores of *Leptographium* for monilial chains of conidia, one would be justified in saying that none of the ambrosia fungi should have been placed in the genus *Monilia*.

Relationship of Leptographium with the Genus Ceratostomella.

Several workers have linked the conidial stage of *Leptographium* with the perfect stage *Ceratostomella*. In 1931, Grosmann (8) described a new species of *Leptographium, L. penicillatum* which she found, together with two or three characteristic yeasts, in constant association with the bark beetle *Ips Typographus* and sometimes with *Pityogenes Chalcographus* in the wood and bark of *Picea excelsa* in Saxon Switzerland and in Würtemberg. She distinguished *L. penicillatum* from *L. Lundbergii* by the form and size of its conidia and by the extreme length of its conidiophore. These features seem comparatively insignificant, particularly as the author herself stressed the variability in size of the conidia. Apart from these characteristics, her cultures agreed very closely indeed with the type species, *L. Lundbergii* as to growth and the manner in which the conidia were borne.

She was able to obtain in culture the perfect stage of the fungus which proved to be a new species of *Ceratostomella* and which she described in a later paper (9) under the name of *Ceratostomella penicillatum*. Single spore cultures from ascospores grew well and gave rise to the typical *Leptographium* stage. The ascospores were oval or slightly curved and measured $6{\cdot}5\mu$ by $2{\cdot}3\mu$, while the figures given for the perithecia were diameter of base, 250 to 300μ, length of neck 300 to 500μ, width of neck about 50μ.

Rumbold (19) studied the relation between bark beetles and blue-stain fungi and in a paper published in 1931 discussed two species of *Ceratostomella* which she found in constant association with these beetles. *Ceratostomella pini* Munch was shown to be constantly associated with *Dendroctonus frontalis* and *D. brevicornis*, and *Ceratostomella ips*, n. sp. with *Ips calligraphus* and *Ips grandicollis*. This new species, *Ceratostomella ips* was described in detail and further points about it given in a later paper (20). Although *Leptographium Lundbergii* was not actually mentioned as the conidial stage, the descriptions and illustrations show a very close resemblance to this fungus. Siemaszko (23) investigating the association of fungi with bark beetles in Poland also assumed that *C. ips* had a conidial stage of *Leptographium Lundbergii*. Describing the formation of the conidia of *C. ips*, Rumbold stated that "those first formed are small, sometimes 2 by 1μ. They are hyaline and obovoid. Later they form on simple conidiophores in a cluster that increases in number as the fungus ages. The conidiophores branch as they grow older until they look like small bushes. In time the bases of the conidiophores turn brown, but the conidia-bearing tips and the conidia themselves remain hyaline. The later conidia range

from 3μ to 10·5 by 1 to 3μ. They are usually clavate." Cultures were at first white but turned warm sepia very rapidly and finally jet black. Perithecia formed in culture were large and long-necked and were 55 to 301μ in diameter with an average of 198μ, 96 to 320μ in height, with an average of 206μ, and the length of the neck varied from 215 to 3,860μ, averaging 1,273μ. Ascospores had the shape of quadrangular prisms and ranged from 2·9 to 4·6μ by 1·2 to 2·8μ with an average size of 3·8μ by 2μ.

Usually there were no bristles at the ostiole of the perithecium although occasionally a few were seen, irregular both in number and length, measuring from 27μ to 45μ. Rumbold remarked that perithecia were formed in the galleries with their bases sunken in the gallery walls and that the beetles kept the necks well trimmed. After the beetles had emerged, the abandoned galleries were often filled with the protruding bristle-like necks of the perithecia.

Rumbold pointed out that the term "association" as used in the descriptions of the connection between *Ceratostomella pini* and *Dendroctonus,* and between *Ceratostomella ips* and *Ips* does not have the significance of the vital association that exists between the ambrosia beetles and the ambrosia fungi. The association appears to be a more casual one for the bark-boring beetles, which are not known to be dependent on fungi for food.

The conidial stage *Leptographium Lundbergii* was attributed by Rumbold (19) to another species of *Ceratostomella, C. piceaperda,* which she found in association with the bark beetle *Dendroctonus piceaperda* on *Picea glauca* in Canada. Perithecia were produced after about five months in culture and the ostioles were without bristles. Ascospores were hyaline and ellipsoid and measured 3·6 to 4·7μ by 1·9 to 2μ with an average of 4·3 by 2μ.

Lagerberg and Melin did not connect their newly-named fungus *Leptographium Lundbergii* with any of the species of *Ceratostomella* but considered it a distinct form. They noted that Falck pictured a fungus exactly similar to it as the conidial stage of *Ceratostomella piceae,* but decided that the author was dealing with a mixture of fungi. *C. piceae* typically has *Cephalosporium* and *Graphium* conidial stages. MacCallum (17) in working with Scottish blue-stain fungi and *Ceratostomella piceae* in particular, also illustrated a branched conidiophore identical with that of *L. Lundbergii,* but he made no mention of it except to note that a mixture of forms was present.

A species of *Ceratostomella* was found by Doane and Gilliland (5) to be associated with the ambrosia beetle *Gnathotrichus sulcatus* on Douglas Fir in California. Two distinct forms, the conidial and perithecial stages, were observed in the galleries

The conidial stage, which was not given a name and was incompletely described was replaced by definite black perithecia after the beetles had left their galleries. The perithecia were flask shaped and smaller at the base. No further morphological details were given and it is not possible to say which species of *Ceratostomella* the authors were examining.

The Association of Ceratostomella with the Australian Ambrosia Fungus.

On examining sections of the tunnels of the Australian ambrosia beetle in the timber of *Nothofagus cunninghami,* a cluster of fine bristles, 35 to 45μ long was seen occasionally to project into the space of the tunnel from the darkened mass of hyphae filling the wood vessels. In a few cases, these bristles were observed to belong to dark elongated perithecia which had formed in the vessels and which contained small asci with eight hyaline rectangular or prism-shaped ascospores (fig. 6). In addition, groups of ascospores were lying freely in the tunnels adjacent to these perithecia. The ascospores measured 5 to 7μ by 3 to 4μ with an average size of 5·9 by 3·5μ. It should be noted that the measurements only relate to a very small number of ascospores and that an average size for the fungus in question could not fairly be taken from them.

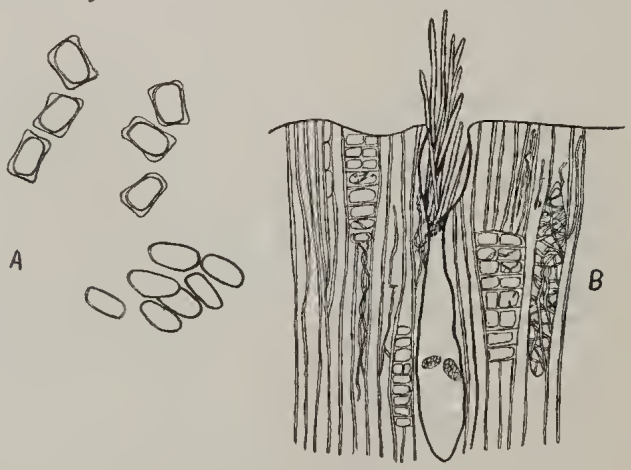

Fig. 6.—A. Ascospores from a beetle tunnel. × 960.
B. Bristles projecting into the tunnel from a sunken perithecium. × 160.

Perithecial measurements could not be made, but the peculiar prism-like shape of the ascospores, the diffluent asci and the presence of bristles associated with sunken perithecia in the tunnels suggest the possibility of an affinity with the species *Ceratostomella Ips* Rumbold. The fact that three species of *Ceratostomella* associated with wood inhabiting beetles have been

described with a conidial stage of *Leptographium*, heightens the probability that the perithecia mentioned above really constitute a stage in the life history of the ambrosia fungus of the Australian *Platypus subgranosus*. Unfortunately all attempts to induce perithecial formation in cultures of *Leptographium Lundbergii* associated with the Australian ambrosia beetles, by the use of strongly acidified media and media rich in carbohydrates have so far been unsuccessful.

Isolation of Yeasts from the Tunnels of Platypus subgranosus.

As already noted two characteristic sporogenous yeasts belonging to the genus *Endomycopsis* Dekker were isolated constantly from the beetle tunnels in Australian timbers together with the ambrosia fungus. The two forms have not been placed specifically but have been designated Forms A and B. Stelling-Dekker (26) published a monograph on the sporogenous yeasts and created the genus *Endomycopsis*, placing in it many species which had formerly belonged to the genus *Endomyces*. The genus *Endomycopsis* is characterized by producing a true mycelium with septa together with yeast cells which show many-sided budding. In the genus *Endomyces* she placed those forms with true mycelium and yeast cells which only divide by transverse fission and not by many-sided budding. In both genera, the ascospores are as a rule hat shaped.

In Form A, asci are produced in whorls at the end of much branched septated hyphae and, after the ascospores have been shed, a new ascus is often seen to grow up inside the old one (see fig. 7 and Plate IV.). The asci are oval in shape and range

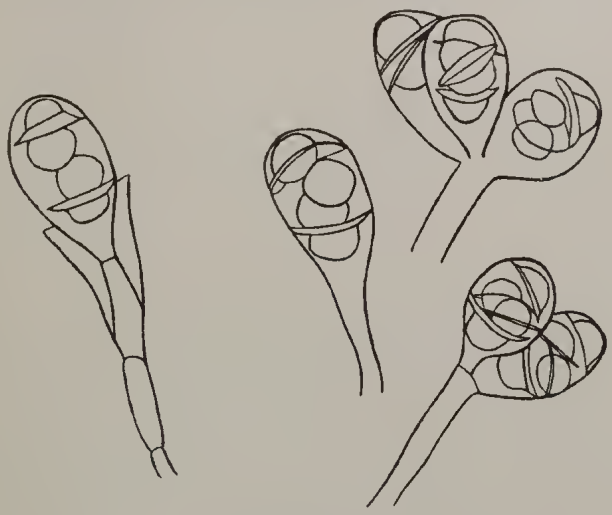

Fig. 7.—Asci and ascospores of Endomycopis sp. Form A. × approx. 720.

from 17·5 to 26μ by 11·5 to 20·3μ, with an average of 21·5μ by 13·9μ. They contain four ascospores which are large and very striking and which are produced readily on malt agar. They are prominently hat shaped, with the flange forming a definite brim. The measurements are as follows:—

	Range.	Average.
Diameter including brim	.. 8–14 μ	11·8 μ
Diameter without brim	.. 5- 8 μ	7 μ
Depth	.. 4- 6 μ	5 μ

Only once has a complete ascus of Form A, with its four large hat-shaped ascospores been seen in the tunnel of the Australian ambrosia beetle in Myrtle Beech, although yeast cells have been very often observed.

The asci in Form B are considerably smaller and are more rounded than those of Form A (fig. 8). They may form in chains

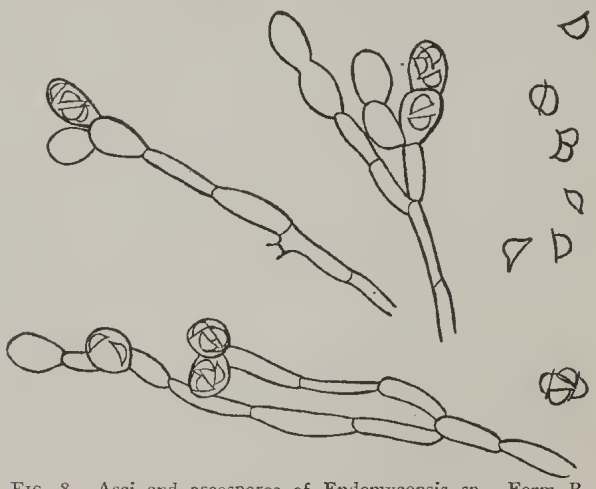

Fig. 8.—Asci and ascospores of Endomycopsis sp. Form B. × 960.

as well as in whorls on the branched septated mycelium. They range from 6 to 9μ by 5 to 6μ averaging 7 by 5·5μ, and they contain four small inconspicuous hat-shaped ascospores. The ascospore measurements are as follows:—

	Range.	Average.
Diameter including brim	.. 3-5 μ	3·8 μ
Diameter without brim	.. 2-3·5 μ	2·9 μ
Depth	.. 1·5-3 μ	2·2 μ

On malt agar, Form A produces a tough, much wrinkled buff-coloured colony with distinctly mycelial edges. In liquid wort, a gelatinous sediment but no pellicle is formed. The colony of Form B on malt agar is white and shining, somewhat wrinkled and fluted in the centre but smooth towards the outside with mycelial edges. With age, the colour becomes greyish. In liquid wort, growth is similar to that of Form A, producing a gelatinous sediment but no pellicle.

The Association of Yeasts with Wood-inhabiting Beetles.

The association of yeasts with wood-inhabiting beetles is of common occurrence and has been reported by many workers.

Schneider-Orelli (22), whose study of the ambrosia fungus of *Xyleborus dispar* has already been mentioned, stated that yeasts were always present in the tunnels of the ambrosia beetles, but he regarded them merely as infections. He did not indicate whether they were always of the same type nor did he give any details.

In 1922, Beck (2), described a new species of yeast, *Endomyces bisporus,* which she found associated with the bark beetle *Ips typographus* on the bark of fir. As the name implies, the ascus only contained two ascospores. Stelling-Dekker transferred this yeast to the new genus *Endomycopsis,* calling it *Endomycopsis bisporus* on the grounds that the yeast cells showed many-sided budding and not just transverse fission. Verrall (3) in 1940 constantly isolated a similar yeast from the tunnels of the ambrosia beetle *Platypus compositus* in pecan, sweet-gum and swamp tupelo, and considered it to be the ambrosia fungus of that beetle. Apparently in ignorance of Beck's earlier work, he called the yeast *Endomyces bispora* n. sp., making no mention of Beck's species of that name or of Dekker's transference of it to the genus *Endomycopsis.*

Siemaszko (23) found members of the Saccharomycetaceae, mostly of a type closely resembling *Endomyces bisporus,* in constant association with *Ophiostoma (Ceratostomella) penicillata* and other species and the bark beetle *Ips typographus* on spruce in different parts of Poland.

Grosmann (8) in her work on the association of bark beetles and blue-stain fungi also isolated yeasts. They were of three types, a budding yeast with hat-shaped ascospores arising parthenogenetically, a second sporogenous one forming mycelium in addition to yeast cells, and a mycelium-forming asporogenous yeast.

Leach, Orr, and Christensen (15) found a characteristic yeast constantly associated with bark beetles and the blue-staining fungi in felled Norway Pine timber. On examining larvae they were often, although not always, able to demonstrate the presence of yeast cells in the intestine, while they were always present in varying amounts in the food contents of the intestinal tracts of freshly emerged beetles. However, the yeast cells did not show any signs of having been digested and used as food.

Studying the association of bark beetles and *Ceratostomella* spp., Rumbold (20, 21) in 1936 and again in 1941, noted that in making cultures from the timbers around the beetle galleries, yeasts were always the first organisms to appear, the blue-stain fungi only developing later. One of these yeasts was described by Holst (12) in a separate paper as *Zygosaccharomyces pini*, a sporogenous yeast forming hat-shaped ascospores but no mycelium. Holst was unable to produce evidence of any direct relationship between the yeast and the beetle. According to Rumbold, the yeasts seemed to have a stimulating effect on *Ceratostomella montium*, causing it to grow more vigorously and to fruit more quickly than in pure culture.

If, as she suggests, the yeasts tend to accelerate growth and sporulation of the fungus, there may be a definite significance in the constant presence of the *Endomycopsis* spp. in the tunnels of the Australian ambrosia beetles. It is conceivable that they stimulate the growth and fruiting of the ambrosia fungus and so increase the food crop for the beetles and their larvae. In addition to this, being rich in protein, they may serve directly as food for the beetles.

Support for this suggestion is to be found in the work of Guyénot (10) who showed that bacteria-free larvae of the fruit-fly *Drosophila ampelophila* may breed entirely on yeast. Under natural conditions, the larvae feed principally on yeasts and other micro-organisms. He reported that he had been able to raise fourteen generations of the fruit-fly in the absence of living organisms. The larvae were reared equally well on potato and living yeast, potato and dead yeast, and on dead yeast alone, but did not grow normally on sterile potato. These results were corroborated and amplified by Baumberger (1) who found that sterile larvae of *Drosophila* lived only five days and showed no increase in size on agar medium containing sugars, mineral salts, and ammonium tartrate as a source of nitrogen, but grew at a normal rate and pupated normally if the medium were infected with living yeasts. The larvae were also able to live on dead yeasts, showing that they were not dependent on the bi-products of fermentation but actually needed the yeasts as food. A concentration of 2 per cent. yeast was sufficient for normal growth. Baumberger concluded that insects inhabiting fermenting and decaying substrata of low protein content usually feed on the micro-organisms present and thus benefit by the power of fungi to extract, absorb, and synthesize many non-protein compounds.

Steinhaus (25), reviewing work on the microbiology of insects, stated that a type of symbiotic feeding on wood and similar substances is presented by the beetle *Anobium paniceum* which has special appendages of the mid-intestine containing Saccharomycetes in their cells.

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Description of Plate.

PLATE IV.

Fig. 1.—Photomicrograph of the fungal palisade lining a beetle tunnel. × approx. 95.

Fig. 2.—Portion of the same palisade under a higher magnification, showing ends of the compact conidiophores. × approx. 400.

Fig. 3.—Asci and hat-shaped ascospores from a culture of Endomycopsis sp. Form A. × approx. 1,800.